REMARKS

Claims 20-22, 31, and 33-47 were pending in the application. Through this paper, claims 20-22, 31, 33-37, 42, and 44-47 have been amended without any intent of disclaiming equivalents thereof. Claim 43 has been canceled without prejudice. New claims 48-58 have been added. Accordingly, upon entry of this paper, claims 20-22, 31, 33-42, and 44-58 are pending and presented for consideration.

Support for amendments to claim 20 can be found throughout the specification, for example, at page 4, lines 4-12 and 15-19; page 5, lines 16-19; page 7, lines 19-31; and page 9, lines 9-14. Support for amendments to claims 21, 22, 33, 34, and 45-47 can be found in the specification, for example, at page 8, line 31. Support for amendments to claim 31 can be found throughout the specification, for example, at page 4, lines 4-12 and 15-19; page 5, lines 16-19; page 6, lines 14-19; and page 7, lines 19-31. Claims 35 and 36 have been amended to claim the correct dependency. Claims 37 and 44 have been amended to correct typographical errors. Claim 42 has been amended to be consistent with amendments made to claim 20. Claims 44-47 have been amended to be consistent with amendments made to claim 31.

Support for new claim 48 can be found throughout the specification, for example, at page 6, lines 21-26 and page 9, lines 9-14. Support for new claim 49 can be found in the specification, for example, at page 5, lines 1-4, and page 9, lines 9-14. Support for new claims 50 and 51 can be found in the specification, for example, at page 13, line 28 to page 14, line 1. Support for new claim 52 can be found in the specification, for example, at page 8, lines 18-24, and page 9, lines 11-14. Support for new claim 53 can be found in the specification, for example, at page 8, lines 1-2. Support for new claims 54 and 58 can be found in the specification, for example, at page 8, lines 12-14. Support for new claims 55 and 56 can be found in the specification, for example, at page 8, lines 1-6. Support for new claim 57 can be found in the specification, for example, at page 9, lines 17-19.

Applicant respectfully submits that the amendments do not introduce new matter and that they are made without any intention to abandon any subject matter as filed.

Claim Objections Under 37 C.F.R. § 1.75(c)

The Office action objects to claims 20, 31, and 43 under 37 C.F.R. § 1.75(c). Claims 20 and 31 have been amended through this paper to address the objections. Claim 43 has been cancelled without prejudice and, as such, the objection with respect to claim 43 is moot. Accordingly, Applicant respectfully requests the objections under 37 C.F.R. § 1.75(c) be reconsidered and withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

The Office action rejects claims 20-22, 31, and 33-47 under 35 U.S.C. § 112, second paragraph. Specifically, the Office action asserts that claims 20 and 31 are unclear by reciting "an amino acid sequence homologous or analogous to the extreme N-terminal SCR-module" without pointing out what functional characteristics must be retained in the claimed polypeptide that is "homologous" or "analogous." Both claims 20 and 31 have been amended to recite "a ligand selected from a group consisting of a polypeptide comprising the entire or essentially the entire protein S binding site in C4b-binding protein (C4BP), and a polypeptide having essentially the same protein S binding properties as C4BP comprising an amino acid sequence homologous or analogous to the protein S binding site of the C4BP molecule." Therefore, Applicant submits that claims 20 and 31, as amended, clearly specify the required functional characteristics of a polypeptide that is homologous or analogous to the extreme N-terminal SCR module of the beta-chain of the C4BP molecule, *i.e.*, having essentially the same protein S binding properties. Accordingly, Applicant respectfully submits that the rejections over claims 20 and 31 have been overcome.

The Office action further rejects dependent claims 37 and 44 for requiring that the ligand be the extreme N-terminal SCR module while independent claims 20 and 31, from which claims 37 and 44 depend, respectively, recite a sequence homologous or analogous to the extreme N-

terminal SCR module. As indicated above, independent claims 20 and 31 have been amended to recite "a ligand selected from a group consisting of a polypeptide comprising the entire or essentially the entire protein S binding site in C4b-binding protein (C4BP) and a polypeptide..." Accordingly, Applicant respectfully submits that amendments to claims 20 and 31 have overcome the rejections of claims 37 and 44.

The Office action alleges that the language "fragment thereof" in claim 20[1] is confusing. Claim 20 has been amended to delete the objected-to language.

The Office action alleges that claim 31 is confusing by claiming a "composition." Claim 31 has been amended to replace "composition" with "kit." Accordingly, Applicant respectfully submits that this rejection has been overcome.

The Office action alleges that claims 34 and 46 recite improper Markush groups because the member "matrix" fails to further define the "solid carrier." Without acquiescing to the rejection and in order to advance prosecution of this application, Applicant has amended claims 34 and 46 to replace "solid carrier" with "carrier," and submits that the amendment overcomes the rejection.

Accordingly, at least in view of the foregoing amendments and remarks, Applicant respectfully requests all rejections of claims 20-22, 31 and 33-47 under 35 U.S.C. § 112, second paragraph, be reconsidered and withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 20-22, 33-43, 46, and 47 are rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the written description requirement. Specifically, the Office action asserts that the language "a fragment thereof" as used in claim 20 is a new matter. Without acquiescing to the rejection and in order to advance prosecution of this application, Applicant has amended

[1] The Office action refers to claim 31, which appears to be a typographic mistake. Applicant bases this response on the foregoing assumption and requests the opportunity to amend this response should the assumption be wrong.

claim 20 to delete the language "a fragment thereof." Accordingly, Applicant respectfully submits that this rejection is moot and requests its withdrawal.

The Office action alleges that the term "bead" recited in claims 33, 34, 46 and 47 improperly broadens the scope of the nature of the bead because the beads are specifically disclosed as being "of polystyrene" and "having a diameter of about 5 mm" in the specification at page 13, line 29. The Office action also alleges that the term "gel" recited in claims 34 and 46 lacks support because no "gel" is taught in the paragraph spanning pages 13 and 14 of the specification.

Applicant respectfully disagrees with the Office's reading of the specification. As set forth on page 13, lines 26-29, the specification states: "Useful solid matrices are well known in the art and are composed of water insoluble materials, such as cross-linked dextran available under the trademark Sephadex from Pharmacia Fine Chemicals; agarose; beads of polystyrene having a diameter of about 1 μm to about 5 mm" (emphasis added). First, "beads of polystyrene having a diameter of about 1 μm to about 5 mm" are disclosed clearly only as exemplary embodiments for illustration purposes. One of skill in the art upon reading Applicant's specification would readily understand that the material and the size of the beads are neither essential nor critical features of the invention. Second, agarose, one of the most common types of gel in the art, is also disclosed as one exemplary embodiment of the invention. One skilled in the art would readily understand from cited portion of the specification that gel is contemplated as an embodiment of the carrier. Finally, the specification teaches cross-linked dextran available under the trademark Sephadex from Pharmacia Fine Chemicals. It is common knowledge in the art that Sephadex, also known as Sephadex beads, is bead-formed gel. An excerpt about agarose gel taken from Molecular Cloning: A Laboratory Manual (Sambrook *et al. eds.*, 1989) and an article published in 1993 relating to Sephadex are attached as Exhibit A and the relevant text is highlighted as evidence of knowledge in the art regarding agarose and Sephadex, respectively.

Therefore, Applicant respectfully submits that (1) the term "bead" recited in claims 33, 34, 46, and 47 is sufficiently supported in the specification and fully in compliance with the

written description requirement and (2) "gel" is also contemplated as an embodiment of the carrier. Accordingly, Applicant respectfully requests these rejections be reconsidered and withdrawn.

The Office action alleges that the term "sheet" as recited in claims 34 and 46 improperly broadens the scope of the nature of "sheets" because the specification only supports "sheets" to the extent of "nitrocellulose- or nylon-based webs." Applicant has amended both claims 34 and 46 to recite that the carrier is "of a structure selected from . . . a sheet, . . ." As set forth on page 13, lines 26-32, the specification states: "Useful solid matrices are well known in the art and are composed of water insoluble materials, such as . . . polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs, such as sheets, strips or paddles." Clearly, the specification teaches that the invention contemplates using various materials to construct the carrier in the structure of sheets. Accordingly, Applicant respectfully submits that the term "sheet" in amended claims 34 and 46 is sufficiently supported in the specification and respectfully requests reconsideration and withdrawal of the rejection.

The Office action alleges that the terms "blood, plasma or serum" used in claim 43 lack support. Claim 43 has been cancelled without acquiescing to the rejection and in order to advance prosecution of this application and, as such, the rejection with respect to claim 43 is moot.

Accordingly, at least in view of the foregoing amendments and remarks, Applicant respectfully requests all rejections of claims 20-22, 33-43, 46, and 47 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 102(a) over Griffin et al.

The Office action rejects claims 20-22, 33-40, and 42 under 35 U.S.C. § 102(a), contending that they are anticipated by Griffin *et al.* (WO 93/01209). Applicant traverses the rejection to the extent it is maintained over the claims as amended.

Anticipation under 35 U.S.C. § 102 requires that all of the elements and limitations of the claims at issue be found within a single prior art reference. Carella v. Starlight Archery and Pro Line Co., 804 F.2d 135, 231 U.S.P.Q. 644 (Fed. Cir. 1986).

Griffin *et al.* does not teach all the elements and limitations of Applicant's claims. Independent claim 20, as amended, recites "a ligand selected from a group consisting of a polypeptide comprising the entire or essentially the entire protein S binding site in C4b-binding protein (C4BP), and a polypeptide having essentially the same protein S binding properties as C4BP comprising an amino acid sequence homologous or analogous to the protein S binding site of the C4BP molecule, the ligand capable of binding a free protein S at a first site" and "a reagent that binds protein S at a site distinct from the first site." (Emphasis added.) Therefore, amended claim 20 requires a ligand and a reagent, both of which bind to protein S at distinct sites. Griffin *et al.* at least does not teach or suggest such a reagent. In contrast, Griffin *et al.* describes a protein S polypeptide or an anti-free protein S antibody that binds immobilized C4BP and competes with free protein S (*see, e.g.*, WO 93/01209, page 52, lines 3-10). Therefore, Applicant submits that Griffin *et al.* does not disclose each and every element of the invention claimed in Applicant's amended claim 20. Accordingly, Applicant respectfully requests reconsideration and withdrawal of rejections of claim 20 and its dependent claims including claims 21, 22, 33-40, and 42 under 35 U.S.C. § 102(a).

Rejections Under 35 U.S.C. § 103(a) over Hardig et al. in view of Griffin et al.

The Office action rejects claims 20-22, 33-40, and 42 under 35 U.S.C. § 103(a), contending that they are unpatentable over Hardig *et al.* (1996) J. Biol. Chem. 271:20861-20867 in view of Griffin *et al.* (WO 93/01209). Applicant traverses the rejection to the extent it is maintained over the claims as amended.

Applicant submits that a combination of disclosures of Hardig *et al.* and Griffin *et al.* would not teach Applicant's invention as claimed in amended independent claim 20. As discussed above, amended claim 20 requires a reagent that binds protein S at a site distinct from

a ligand-binding site. Neither Hardig *et al.* nor Griffin *et al.* teaches or suggests a reagent that binds protein S at a site distinct from a ligand-binding site. Therefore, even if the disclosures of Hardig *et al.* and Griffin *et al.* were combined, such a combination would not teach Applicant's claimed invention. Applicant therefore respectfully requests reconsideration and withdrawal of the rejection of claim 20 and its dependent claims including claims 21, 22, 33-40, and 42 under 35 U.S.C. § 103(a).

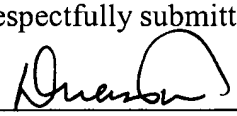
REQUEST FOR TELEPHONIC INTERVIEW

Applicant respectfully requests a telephonic interview in order to expedite the prosecution of the claims. The Examiner is invited to telephone the undersigned at 617-248-7808 to arrange a convenient time to discuss any outstanding issues, and to work with the Examiner toward placing the application in condition for allowance.

CONCLUSION

Applicant respectfully urges that all claims are in condition for allowance and requests prompt and favorable action on this application.

Respectfully submitted,



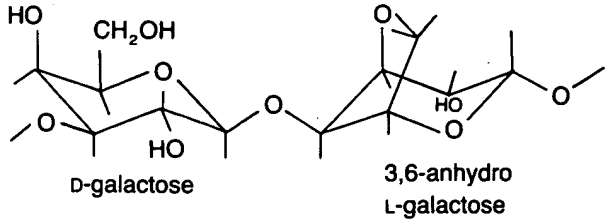
Date: October 15, 2004
Reg. No.: Limited Recognition

Duan Wu
Attorney for Applicant
Testa, Hurwitz, & Thibeault, LLP
High Street Tower
125 High Street
Boston, Massachusetts 02110

Tel. No. (617) 248-7808
Fax: (617) 248-7100

Agarose Gel Electrophoresis

Agarose, which is extracted from seaweed, is a linear polymer whose basic structure is



Commercially available agarose is not completely pure; it is contaminated with other polysaccharides, salts, and proteins. The amount of contamination varies from batch to batch of agarose and from manufacturer to manufacturer. These differences can affect both the migration of the DNA and the ability of the DNA recovered from the gel to serve as a substrate in enzymatic reactions. Because of the great increase in demand during the past 10 years, most manufacturers now prepare special grades of agarose that are screened for the presence of inhibitors and nucleases and for minimal background fluorescence after staining with ethidium bromide.

Some manufacturers also sell chemically modified forms of agarose that gel and melt at low temperature without significant deterioration in the strength of the hardened gel. Such chemically modified agaroses are used chiefly for preparative electrophoresis of DNA and for digestion of DNA with restriction enzymes in situ. Special grades of low-gelling-temperature agarose that can be used to analyze very small fragments of DNA (10–500 bp) are also available from some manufacturers. Gels made with agarose of this type have a greater resolving power than gels made with normal agarose, but the resolution obtained from these gels still cannot compare with the resolution obtained from polyacrylamide gels. Furthermore, since these gels contain a high concentration of agarose (4–10%), DNA fragments eluted from the gel are frequently contaminated with inhibitors that prevent further enzymatic manipulation.

Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved. The melted solution is then poured into a mold and allowed to harden. Upon hardening, the agarose forms a matrix, the density of which is determined by the concentration of the agarose. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates toward the anode. The rate of migration is determined by a number of parameters, which are discussed on the following pages.

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ACTIVITIES EXCHANGE

SEPARATION OF A STARCH-GLUCOSE MIXTURE USING GEL FILTRATION

Fred Blumenfeld
1993 Woodrow Wilson Biology Institute

INTRODUCTION

For more than thirty years, gel filtration has been used as a biotechnological process for the purification of enzymes, polysaccharides, nucleic acids, proteins and other biological macromolecules. The techniques of gel filtration separate molecules according to differences in their size as they pass through a column packed with a gel media. Gel filtration media are very stable because of their ineptness towards biopolymers. In the experimental laboratory, gel filtration is reliable and simple, little equipment is required, the procedures are straightforward and good separations and yields are usually obtained.

Several years ago, I began experimenting with a number of gels to determine the feasibility of designing high school laboratory experiences using gel filtration. I have successfully completed and utilized a number of these labs in my classroom. One of these is the separation of a starch-glucose mixture using gel packed PD-10 columns.

The gel used, Sephadex G-25, can be purchased pre-packed in polypropylene syringe-like PD-10 columns. The gel is an inert, bead-formed, cross-linked Dextran polymer consisting of many glucose molecules. Sephadex beads are porous. Molecules larger than the largest pores cannot enter the gel and are eluted (pass out) from the column first. Smaller molecules enter the beads and are retarded based on their size. Therefore, molecules are eluted in order of decreasing size. Sephadex G-25 excludes all molecules with a molecular weight greater than 5000, thereby eluting them first.

The following concepts developed are:

1. Starch is a larger molecule than glucose
2. An assay based upon selection
3. The techniques of testing for the presence of specific substances
4. The basic principles of gel filtration

TARGET AGE/ABILITY GROUP:

A maximum of twenty four students in grades 9-12 of average to high ability can be targeted for each class instruction period.

CLASS TIME:

Forty-five minutes to one hour should be scheduled as operating time, depending on prior preparation.

MATERIALS:

For team of two students

- Test tube rack to hold 18 small test tubes, 13 x100 mm.
- Two beakers
- Wire test tube holder.
- Masking tape
- One ring stand with burette clamp.
- Water bath
- One PD-10 gel column G-25.
- Hot plate
- 0.1% starch and glucose mixture - in dropping bottle
- 0.9% sodium chloride solution - 30 ml in Barnes dropping bottle
- Benedict's solution in dropping bottle
- Lugol's iodine solution in dropping bottle
- Razor blades, single edge

Materials should not exceed twenty dollars. I have used and reused the PD-10 columns for at least 5 years.

SAFETY PRECAUTIONS:

Wearing of goggles, use of test tube holders, use of heat resistant gloves, aprons and disposal of end products in evaporating containers placed in hoods.

TEACHER GUIDE

Preparation time need not exceed one hour including preparation of solutions which follow.

1. Starch-glucose mixture.

   Weigh out 1 gm of soluble starch and 1 gm of glucose (dextrose) and place in a two liter beaker. Add one liter of distilled or deionized water and heat to boiling while stirring. Let cool before using.

2. 0.9% sodium chloride

Weigh out 9 gms of sodium chloride and add to 991 gms (about a liter) of distilled or deionized water.

3. Lugol's iodine solution

   This can be purchased from any biological or chemical supply house, or it may be made by dissolving 5.0 grams of iodine plus 10.0 grams of potassium iodide in 100 ml of distilled or deionized water and used as a stock solution. The stock solution should be diluted one to ten parts of water before using.

4. Benedict's solution

   This is best purchased from a biological or chemical supply house.

5. PD-10 Columns Prepacked with gel, and swollen in 0.9% salt solution.

   Can be purchased from:
   Pharmacia Fine Chemicals
   800 Centennial Avenue,
   Piscataway, New Jersey 08855-1327
   1-800-526-3593

   PD-10,30/pk. No. 17-0851-01 $112.00

TEACHERS OUTLINE FOR PRESENTATION OF ACTIVITY

1. Discuss concepts presented in introduction.
2. Review safety precautions.
3. Present flowchart of procedure
4. Model data collecting technique.
5. Observe these helpful hints to avoid sources of error:

   a. Step 3 in student procedure pertaining to elution of sodium chloride is to ensure that glucose from previous class usage will be removed from column in case students forgot to completely elute the glucose.

   b. It is important that when filling the column with salt solution for collecting of fractions, there is no overflow into the collecting test tubes.

   c. An alternative method of numbering test tubes is to label the rack openings directly using a strip of masking tape. Be sure all test tubes are thoroughly cleaned.

d. Make certain that students do not misplace top and spout caps.

ANSWERS TO QUESTIONS

1. Student responses.

2. The first 10 drops contained no starch or glucose, as the remainder of the salt in the column was still eluting through. The starch comes into test tubes 2 and 3, as the starch molecules are much larger than the glucose molecules and cannot penetrate the gel beads. Test tube #4 may indicate a slight presence of both starch and glucose as that fraction may contain the last part of the starch solution, and the initial part of the glucose solution or glucose may be absent showing complete separation. Only test tubes #5-8 should show increasing, then decreasing concentrations of glucose, with no starch being present. Test tube #9 and #10 are negative for both starch and glucose since neither substance is present in the column, and only salt is coming through.

3. This technique differs from dialysis in that the gel process can be considered in two ways. If you view the entire column as the membrane, then only the glucose should pass through, starch should be left behind- which obviously is not the case. If you view each gel bead as the membrane, there is no difference.

4. Sodium chloride elutes the solutes through the gel column as well as cleansing the gel column at the conclusion of the lab. Sodium chloride also keeps the concentration of the salt the same both inside and outside the beads.

5. Ten drops are collected first, as the bottom of the column contains only salt. Salt comes through the gel column with the starch. This could be proven by adding lead nitrate to any fraction collected, and obtaining a white precipitate.

6. Purification of biological macromolecules e.g. proteins, nucleic acids, photosynthetic pigments, inorganic mixtures.

OTHER

Another biological experiment that uses the principle of gel filtration is the separation of pigments from Coleus leaves. The gel, LH-20, is required. The organic solvent, 95% Ethanol separates the extract into distinct bands of chlorophyll, xanthophyll and anthocyanin.

SOURCES OF MATERIALS AND REFERENCES

Carolina Biological Supply Co.
Burlington, North Carolina
Kemtec Co. Cincinnati, Ohio

Pharmacia Fine Chemicals
Pharmacia LKB Biotechnology Manual
"Principles and Methods of Gel Filtration"

James Gardner, Consultant,
Fairleigh Dickinson University,
C.E. Div. Madison, NJ

SEPARATION OF STARCH FROM GLUCOSE USING GEL FILTRATION

PURPOSE:

To separate starch from glucose in a mixture.

MATERIALS:

For team of two students

- Test tube rack to hold 18 small test tubes, 13 x100 mm
- Two beakers
- Wire test tube holder
- Masking tape
- One ring stand with burette clamp
- Water bath
- One PD-10 gel column G-25
- Hot plate
- 0.1% starch and glucose mixture - in dropping bottle
- 0.9% sodium chloride solution 30 ml in Barnes dropping bottle
- Benedict's solution in dropping bottle
- Lugol's iodine solution in dropping bottle
- Razor blades, single edge

State a hypothesis concerning the outcome of this experiment based on background information.

PROCEDURE:

1. Mount gel column vertically with burette clamp.

2. Number test tubes 1S through 8S and 1G through 10G ("S" is for Starch and "G" is for Glucose) and place in test tube rack.

3. Allow eluant (a substance used to remove any material already present in the gel column) to drip out into beaker until flow stops. Add 1.0 ml or 20 drops of 0.1% glucose and starch

mixture to the top of the gel column, continuing to collect eluant in the beaker. Discard all eluant washing.

4. When eluant flow has stopped, cap outlet spout.

5. Add 0.9% salt solution to fill top of column, and lower the gel column so that the spout is about 2.0 cm above test tube #1S.

6. Remove outlet spout cap, and allow 10 drops to enter test tube #1S.

7. Moving the test tube rack, collect an additional 25 drops in each test tube numbered 2S through 8S. Collect an additional 10 drops in test tube 9G and 10G. The column will have to be refilled at least twice with 0.9% sodium chloride during this part of the lab.

8. Cap the outlet spout after test tube #10G has been collected. Also make certain that the space above the gel column is only about 1/2 filled with 0.9% sodium chloride solution before replacing the top cap.

9. In test tubes #1G through 10G place one ml or 20 drops of Benedict's solution.

10. Pour all but a few drops of eluant collected in test tube #1S into test tube #1G containing the Benedict's solution. Similarly, repeat pouring eluants from test tube #2S into test tube #2G, test tube #3S into test tube #3G, . . . through test tube #8S into test tube #8G. This effectively divides the eluant of each test tube numbered 1S through 8S into two parts. The largest portion, in test tubes numbered 1G through 8G will be used to test for glucose. In addition test tube #9G and 10G will be tested for glucose. The other portion, containing only a few drops, in test tubes numbered 1S through 8S will be used for starch identification.

11. Place one drop of iodine solution into each test tube numbered 1S through 8S containing a few drops of eluant. Observe results for starch identification (blue-black color) by holding against a white background and note observation in data chart as follows: (++) for intense color change (+) for slight color change (-) for a negative test

12. Gently heat contents of test tubes numbered 1G through 10G in water bath for a few minutes or until any color change occurs. Observe color changes, if any, in each test tube for glucose identification, and note observations in data chart as follows: (++) for a dark green to brick red (+) for a yellow to light green (-) for a blue color

13. Clean all glassware thoroughly.

TEST TUBES	1S	2S	3S	4S	5S	6S	7S	8S
Starch Id.	*	*	*	*	*	*	*	*

TEST TUBES	1G	2G	3G	4G	5G	6G	7G	8G	9G	10G
Glucose Id.	*	*	*	*	*	*	*	*	*	*

CONCLUSIONS:

Answer the following questions.

1. Was your hypothesis correct? What evidence do you have to support your hypothesis?
2. Explain your results.
3. How does dialysis differ from this technique?
   How is it similar?
4. What role does the sodium chloride solution play in this experiment?
5. In sample 1S, why were only 10 drops collected? What eluant came through the gel column with the starch, and with the glucose? How could you prove this?
6. What other scientific uses can gel filtration have?

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